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Assessment of pollen viability and germination in *Swainsona formosa* (G.Don) J.Thompson

Mengukur viabilitas serbuk sari dan sukrosa terhadap perkecambahan Swainsona formosa (G.Don) J.Thompson

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Abstract, this study to investigate the proper time (after spreading) for viability assessment and the effect of various sucrose concentrations on pollen germination in *Swainsona formosa*. The rate of pollen tube formation was determined for freshly shed pollen grains of glasshouse-grown plants at 10, 15, 20, 25, 30, 60 and 120-minute intervals after being plated on Brewbaker and Kwack (BK) medium. The results indicated that within 60 minutes pollen germination reached 63.70%, after which remained steady at 120 minutes (63.71%). Under the effect of various concentrations of sucrose, i.e. 0.5, 10, 15, and 20% (w/v), the germination rate of pollen grains was assessed at 60 minutes following germination. The results showed that sucrose concentration of 10 – 15% (w/v) produced better germination rate (64.14%) compare to lower concentrations (19.64 and 43.58% at zero and 5% sucrose, respectively). Sucrose concentration above 15% was also found to inhibit pollen germination (48.92% at sucrose concentration of 20%).

Keywords: desert pea, ornamental plant, plant breeding, legume, Fabaceae.

Abstrak, Percobaan ini bertujuan untuk mendapatkan waktu yang paling tepat (setelah penaburan) untuk mengukur viabilitas serbuk sari, dan untuk mengetahui pengaruh berbagai tingkat konsentrasi sukrosa terhadap perkecambahan serbuk sari *Swainsona formosa.* Laju pembentukan buluh serbuk sari ditentukan pada interval 10, 15, 20, 25, 30, 60 dan 120 menit terhadap serbuk sari segar yang diperoleh dari tanaman di rumah kaca dan dikulturkan pada medium Brewbaker dan Kwack (BK). Hasil percobaan menunjukkan bahwa dalam waktu 60 menit jumlah serbuk sari yang berkecambah mencapai 63,70%, yang kemudian berada pada laju yang tetap (63,71) setelah 120 menit. Di bawah pengaruh berbagai tingkat konsentrasi sukrosa, yakni 0,5, 10, 15 dan 20% (b/v), laju perkecambahan serbuk sari diukur setelah 60 menit penaburan. Hasilnya menunjukkan bahwa konsentrasi sukrosa antara 10 hingga 15% (b/v) menghasilkan laju perkecambahan yang lebih baik (64,14%) dibandingkan konsentrasi yang lebih rendah (19,64 dan 43,58% pada konsentrasi sukrosa nol dan 5%). Sementara itu, konsentrasi sukrosa di atas 15% cenderung untuk menghambat perkecambahan serbuk sari (48,92% pada konsentrasi sukrosa 20%).

Kata kunci: desert pea, tanaman hias, pemuliaan tanaman, legum, Fabaceae.

INTRODUCTION

Swainsona formosa (G.Don) J.Thompson [syn. *Clianthus formosus* (G.Don) Ford *et* Vickery] which has the chromosome number of 2*n* = 16 (Zulkarnain *et al.*, 2002) belongs to the subfamily Papilionoideae of the family Fabaceae. It grows mainly in arid regions of southern and central Australia in South Australia and also occurs in New South Wales, the Northern Territory and Western Australia, particularly on alkaline soils of areas receiving extra water, such as creek or lake margins (Zulkarnain, 2003). Growing naturally, *S. formosa* grows as a prostrate annual or short-lived perennial (Corrick and Fuhrer, 2009) with long trailing stems radiating up to 2 m from the main woody root on sandy or loamy soils.

Swainsona formosa is a temperate plant, therefore it is believed that they can be grown and developed in certain part in Indonesia, particularly in the area of dry lowland (Zulkarnain, 2003). Plants grow best in full sun and flowering is not affected by daylength (Williams, 1996) but light intensities of 600 – 950

µmol m^{-2} s^{-1} are required for flowering (Kirby, 1996b). The optimum temperature is 32.5 ± 2.5 ^{o}C, and plants will grow slowly if daily temperature is below 20 ^{o}C (Kirby, 1996a). Further, (Yusuf *et al.*, 2002) suggested that plants grown under temperature regimes of 18/30 ^{o}C (night/day) produced more flowers than those grown under 10/22 ^{o}C (night/day) or a constant temperature of 25 ^{o}C.

The economic importance of *S. formosa* is in its potential use in a hanging basket or container plant, or as a cut flower plant (Williams and Taji, 1991; Kirby, 1996b; 1996a). However, the commercialization of *S. formosa*, however, is hampered by the production of large amount of pollen grains, which may stains the petals when the anthers dehisce, resulting in poor flower quality. Post-harvest self pollination may also occur during transportation, causing quick flower degeneration and reduction in vase-life. Therefore, the breeding programmed of this species should be focused on the crossing effort to produce hybrids with less or non-viable pollen grains. One of essential requirement in crossing is to have a method of testing pollen viability with a reasonable level of accuracy. We report here the study of pollen viability as an initial step in breeding of *S. formosa.*

MATERIALS AND METHODS

Stock plants establishment

Plant materials were routinely grown on sand and peat (3:1) medium in a glasshouse with an average temperature of 25 – 32 ^{o}C, light intensity of 650 – 1200 µmol m^{-2} s^{-1}, and photoperiod of 12 – 16 hours. Plants were watered once a day using an automatic watering system. Approximately 250 mL supplementary liquid fertiliser with trace elements, Hortico™ Aquasol™ (23% N, 4% P, 8% K, 0.05% Zn, 0.06% Cu, 0.0013 Mo, 0.15% Mn, 0.06% Fe and 0.11% B), was applied weekly at a concentration of 1 g L^{-1}. After the plants had been four weeks in the new pots, a fungicide (Fongarid® 250WP with active ingredient 25% furalaxyl) was applied at a concentration of 1 g L^{-1} to eliminate root diseases such as *Phytopthora* sp. A slow release fertiliser, Nutricote® (16% N, 4.4% P, 8.3% K and 4% Ca), was applied at the time of transplanting and after 8 weeks of transplanting at a concentration of 2 g per pot for each application to ensure optimum nutrient supply. A miticide, Omite® 300W (active ingredient 30% propargite), was applied at the rate of 1 g L^{-1} to kill the spider mite (*Tetranychus* sp.) as required. In addition, an insecticide Clear White Oil (active constituent 820 g L^{-1} petroleum oil) was applied at 0.2% (v/v) to kill mealy bug.

Pollen germination

Flowers on the second day of anthesis were selected from 5 different stock plants. Dry mature pollen grains from each individual flower from 3 – 4 umbels were collected in a small glass vial and mixed thoroughly. Pollen viability and rate of pollen tube formation were tested at different times, i.e. 5-10, 11-15, 16-20, 21-25 and 26-30 minutes after being plated. Pollen germination was undertaken on BK medium (Brewbaker and Kwack, 1963) containing 720 ppm $Ca(NO_3)_2 \cdot 4H_2O$, 200 ppm $MgSO_4 \cdot 7H2O$, 200 ppm KNO_3, 20 ppm H_3BO_3 supplemented with 10% sucrose. Using a paintbrush, pollen was dusted onto and spread evenly over the surface of one drop of this medium on a microscope slide. The slides were placed in closed Petri dishes on a layer of moistened filter paper. The dishes were kept at room temperature (25 ± 1 ^{o}C) and light intensity of 50 µmol m^{-2} s^{-1} to allow the pollen to germinate.

The examination of pollen viability was carried out according to the protocol of Kearns and Inouye (1993). Pollen grains were dusted on a drop of lactophenol-aniline blue on a microscope

slide, covered with a cover slip and examined under a light microscope. Dark blue stained pollen grains were recorded as viable, whereas pale blue stained and colorless grains were classified as unviable.

The assessment of pollen viability was done using aniline blue staining technique according to the protocol of Kearns and Inouye (1993). Pollen grains were dusted on a drop of lactophenol-aniline blue on a microscope slide, covered with a cover slip and examined under a light microscope (Zeiss Standard-20). The viable pollen grains will develop pollen tubes and absorb more blue stain, and therefore, they are darker in colour. In contrast, unviable pollen grains will not develop pollen tubes and absorb less blue stain, resulting in lighter colour. A pollen grain was considered germinated when the length of pollen tube was at least equal to grain diameter (Khan and Perveen, 2008).

Sucrose application

In this trial pollen grains were collected in the same way as the previous trial, germinated on the same medium composition but with different sucrose concentrations, i.e. 0, 5, 10, 15 and 20% (w/v), and kept under similar environmental conditions as the previous experiment. The observation on pollen tube growth was done following 60 minutes of plating.

These trials were arranged in a completely randomised design with 5 replicates. Each replicate consisted of 10 fields of view. The percentage of germinated pollen was determined by counting the number of pollen grains forming tubes divided by all pollen grains seen in one field of view at a magnification of 400x using a Zeiss Standard-20 light microscope. For both experiments only pollen grains with tube formation at least half the diameter of the pollen grain were counted.

RESULTS AND DISCUSSION

Rate of pollen tube formation

The result of *in vitro* germination attempted on BK medium (Brewbaker and Kwack, 1963) containing 10% (w/v) sucrose is presented on Figure 1. Pollen tubes started to grow within the first 10 minutes of culture (28.3% pollen tube formation) but the length of most pollen tubes was less than half of the respective pollen diameter. Tube formation increased dramatically within the next 10 minutes (48.84%) and remained steady after 60 and 120 minutes, after the pollen was initially spread (63.70 and 63.71% pollen germination, respectively). Based on these results, pollen germination on the next trial was assessed at 60 minutes after culture initiation.

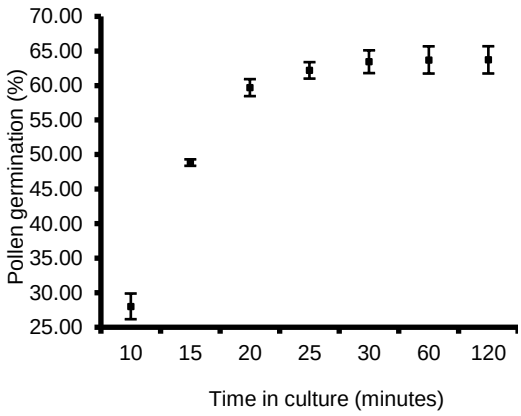

Figure 1. The percentage of germination of *S. formosa* pollen at various times after culture initiation. Bars indicate standard deviations of five replicates.

The use of aniline blue was proven to be useful in determining viable or unviable pollen grains in *S. formosa*. The work presented here showed that viable pollen grains absorbed more stain along with the development of pollen tubes. On the other hand, unviable pollen grains absorbed less stain and did not develop pollen tube (Figure 2). There are various methods of pollen viability test, such as fluorochromatic reaction, Feulgen staining (Prakash, 2000), iodine

potassium iodide, tetrazolium (Sulusoglu and Cavusoglu, 2014) and aniline blue staining (de Jesus Viera *et al.*, 2015). However, there is no universal viability test that reliable for all plant species.

The use of different colorants to test pollen viability may give comparable results (Ilgin *et al.*, 2007; Abdelgadir *et al.*, 2011; Gaaliche *et al.*, 2013; Melloni *et al.*, 2013). Treating the pollen grains with non-vital stains such as aceto-carmine, iodine in potassium iodide, and aniline blue in lactophenol essentially imparts colour to the contents of the pollen in fresh as well as fixed/dead pollen. Our results showed that aniline blue could be reliably used to determine the viability of *S. formosa* pollen grains.

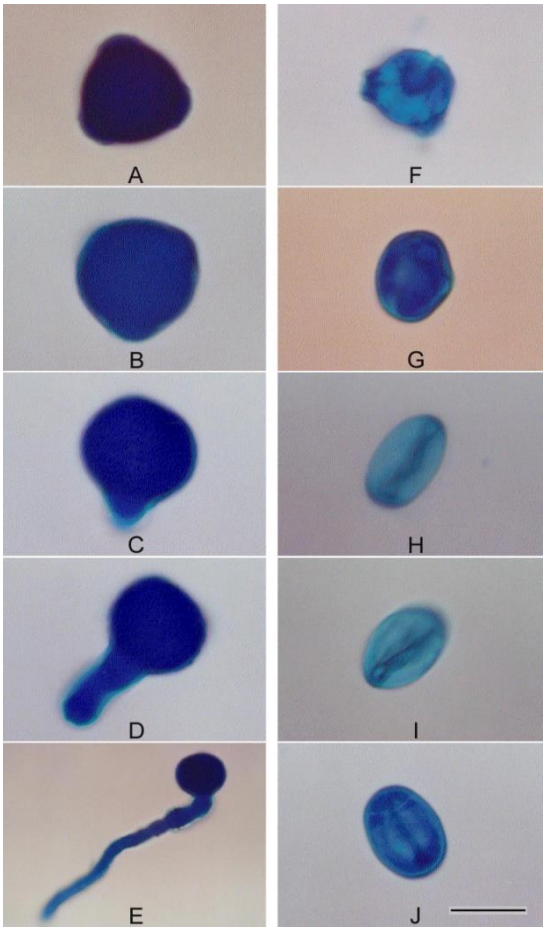

Figure 2. The growth of pollen tube from viable pollen on BK medium supplemented with 10% sucrose. A, pollen grain at the time of culture initiation; B = 10 minutes after germination; C = 20 minutes after germination; D = 30 minutes after germination; E = 60 minutes after germination; F-J = examples of unviable pollen grains. Bar A-D and F-J = 10 µm, E = 25 µm.

The effect of sucrose concentration on pollen germination

With sucrose concentrations of 10 – 15% (w/v), pollen tubes started to grow just within a few minutes after being plated, indicated by a swelling germinal pore (Figure 2B). Following 10 minutes of germination, the length of the tubes reached one-third pollen diameter (Figure 2C). Within the next 30 minutes the rate of tube growth increased rapidly and most pollen produced a tube length approximately 1.5 times the pollen diameter (Figure 2D). About sixty minutes after germination most pollen tubes reached a length of 5 – 6 times the pollen diameter (Figure 2E).

Analysis of variance on the effect of sucrose on *in vitro* pollen germination showed that the number of germinating pollen grains was highly dependent on the presence of sucrose in the media ($P < 0.01$). Further analysis using Fisher's Protected Least Significant Different (FPLSD) test (Petersen, 1985) indicated that the medium with 10% (w/v) sucrose produced the highest percentage of pollen germination (64.14%), which was not significantly different from the 15% sucrose medium that produced 62.91% pollen germination. A further increase in sucrose concentration of up to 20% (w/v) resulted in a significant reduction in germination (48.92%). Significantly lower percentages of pollen germination (19.64 and 43.58%) were also found with sucrose concentrations of 0 and 5% (w/v), respectively. The relationship of various concentrations of sucrose and the rate of pollen germination in *S. formosa* under *in vitro* condition is presented in Figure 3.

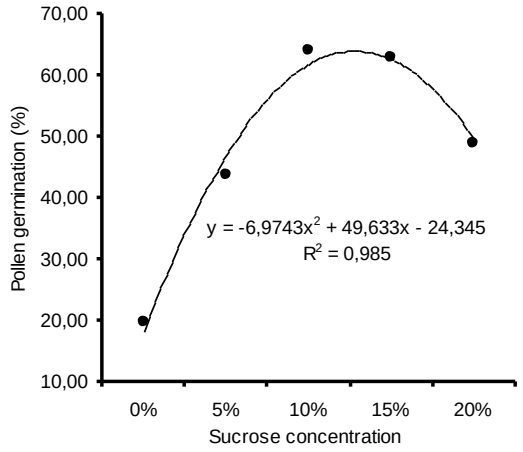

Figure 3. The relationship of various sucrose concentrations and the rate of pollen germination in *S. formosa*.

These responses are comparable to the report of Werner and Chang (1981) and Parfitt and Almehdi (1984) on peach. Further, Polito and Luza (1988) reported that there was no differences in germination of fresh pollen on 10%, 15% or 20% sucrose on *Pistachio vera*. However, with time, 20% sucrose consistently support higher pollen germination level on this plant. Similar results were also reported by (Ilgin *et al.*, 2007) on caprifig, and by Demir *et al.* (2015) on *Citrus* spp. where the optimum sucrose concentrations for pollen germination were 20% and 25%. Their finding was somewhat contradict with the present result, where the rate of *in vitro* pollen germination on *S. formosa* significantly dropped on 20% sucrose. It is clear that no one test is suitable for testing viability in all species.

Conclusion

Based on the results of this study, it can be concluded that: 1) the proper time to determine pollen viability under *in vitro* condition in *Swainsona formosa* is 60 minutes of pollen culture, and 2) sucrose concentration of 10 – 15% (w/v) is recommended to be incorporated in germination medium in pollen viability test.

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